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Formulation-dependent toxicokinetics explains differences in the GI absorption, bioavailability and acute neurotoxicity of deltamethrin in rats

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Abstract

The acute neurotoxicity of pyrethroid insecticides varies markedly with the dosage vehicle employed. The objective of the present study was to assess the influence of two common vehicles on the bioavailability and toxicokinetics (TK) of a representative pyrethroid insecticide, deltamethrin (DLM), to determine whether the vehicles influence toxic potency by modifying the chemical's TK. Adult, male Sprague–Dawley rats were administered DLM iv or po, either by dissolving it in glycerol formal (GF) or by suspending it in Alkamuls® (AL). Groups of rats received 10 mg DLM/kg by gavage in each vehicle, as well as 2 mg/kg in GF or 10 mg/kg in AL by iv injection. Serial blood samples were collected over 96 h and analyzed for their DLM content by HPLC. In a second experiment, plasma, brain, fat, liver and lung DLM concentrations were measured 2 h after giving 10 mg DLM/kg orally in GF or AL. In a third experiment rats received 2 or 10 mg DLM/kg iv in AL or 2 mg DLM/kg iv in GF. Lung DLM content was determined 15 min post injection. DLM particle size in both formulations was measured under a phase contrast microscope. DLM appeared to be completely dissolved in GF, while particle size ranged from <5 to >50 μm in AL. The bioavailability of DLM in the aqueous AL suspension was ∼9-fold lower than in GF (1.7% versus 15%). Blood C_{max} (0.95 ± 0.27 versus 0.09 ± 0.01 μg/ml) and AUC_0^{48h} (5.49 ± 0.22 versus 0.61 ± 0.14 μg·h/ml) were markedly higher in the GF gavage group. Tissue DLM levels were also significantly higher in the GF animals at 2 h. The 10 mg/kg po and 2 mg/kg iv doses of DLM in GF produced moderate salivation and slight tremors. Rats receiving the insecticide in AL were asymptomatic. IV injection of the AL suspension resulted in trapping of much of the dose in the pulmonary capillaries. As anticipated, the injected suspension had a longer half-life and slower clearance than did the GF formulation. In summary, limited dissolution of the highly lipophilic DLM particles in the AL suspension severely limited DLM's GI absorption, bioavailability, target organ deposition and acute neurotoxic potency.

Keywords: Acute neurotoxicity; Deltamethrin; Internal exposure; Pyrethroid; Vehicle effect

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1. Introduction

Pyrethroids have been used for more than 30 years for control of insects in agriculture, public health and the home (ATSDR, 2003). By the mid 1990s, pyrethroid use had grown to represent 23% of the U.S. dollar value of the worldwide insecticide market (Casida and Quistad, 1998). That percentage share and the number of human incidents have continued to increase substantially in the U.S. during the last several years with the declining use of organophosphates (Sudakin, 2006). It was estimated by the ATSDR (2003) that 1 million pounds of permethrin, the most popular pyrethroid, were applied in the U.S. in 2001. Occupational (Vijverberg and van den Bercken, 1990; Soderlund et al., 2002) and nonoccupational (Berkowitz et al., 2003; Heudorf et al., 2004; Lu et al., 2006) exposures of wide segments of the general population to pyrethroids have been well documented. Nevertheless, knowledge of their mechanism(s) of action and long-term toxicity is limited. Information on their toxicokinetics (TK) is even more limited.

Traditionally, pyrethroids are divided into two classes, based upon their chemical structure and clinical manifestations of acute exposure. Type I compounds do not contain a cyano moiety, while Type II do. Hyperexcitation, tremors and skin parathesias are the most common signs of acute poisoning by Type I compounds. Type II pyrethroids' "hallmark" effects are salivation, tremors and choreoathetosis (Lawrence and Casida, 1982; Ray and Forshaw, 2000). Voltage-sensitive sodium channels in central nervous system neurons appear to be their principal site of action (Narahashi, 1996). Deltamethrin (DLM), the compound chosen for the current study, is one of the most potent pyrethroids *in vitro* (Choi and Soderlund, 2006) and *in vivo* (Wolansky et al., 2006). Rickard and Brodie (1985) find that the onset and severity of signs of DLM poisoning in mice are proportional to brain levels of DLM. Additional evidence that the parent pyrethroid is the primary toxic moiety is furnished by Lawrence and Casida (1982). They report that intracerebral injection of DLM produces signs of poisoning in mice within a min or less. It is also reported that inhibitors of enzymes that metabolize pyrethroids exacerbate their toxicity (Soderlund and Casida, 1977; Casida et al., 1983).

It has been recognized for almost 30 years that biotransformation of pyrethroids occurs primarily via two pathways: hydrolysis of the molecules' ester linkage by esterases; and aromatic hydroxylation by cytochrome P450s, with subsequent conjugation (Ruzo et al., 1978; Rickard and Brodie, 1985). Anand et al. (2006a) recently found that carboxylesterases (CaEs) in rat liver and plasma, as well as rat liver cytochrome P450s 1A1, 1A2 and to a lesser degree 2C11 are primarily responsible for DLM biotransformation *in vitro*. Intrinsic clearance by plasma CaEs is far less important quantitatively than metabolism in the liver. Anand et al. (2006b) also found that limited capacity of these enzymes contributes significantly to increased systemic exposure and neurotoxic effects in immature rats.

The number of comprehensive TK studies of orally-administered pyrethroids in animals is very limited. Our laboratory (Kim et al., 2007) has delineated the time-course of DLM in the blood and a variety of tissues of adult rats gavaged with DLM in glycerol formal (GF). Mirfazaelian et al. (2006) utilized these data to develop a physiologically-based pharmacokinetic model for DLM. Anàdon and his colleagues used GF as an iv injection vehicle, but a vegetable oil for oral administration of permethrin (1991), DLM (1996) and lambda-cyhalothrin (2006) in their TK studies.

Oral dosage vehicles can have a marked effect on the acute toxicity of pyrethroids. Published acute LD_{50} values for most pyrethroids given orally to rats in corn or other vegetable oils range from 50 to 500 mg/kg (Soderlund et al., 2002). These authors cited LD_{50} values for lambda-cyhalothrin of 79 and 56 mg/kg when given in corn oil, versus 299 and 433 mg/kg when given as an aqueous suspension. Oral LD_{50} values listed for DLM ranged from 87 mg/kg (in corn oil) to >5000 mg/kg (in a 1% methylcellulose aqueous suspension). Pham et al. (1984) reported that oral DLM was 100 times less toxic to rats when suspended in gum Arabic solution than when dissolved in GF. Crofton et al. (1995) assessed the ability of DLM to depress motor activity of rats gavaged with DLM in four different vehicles. The chemical was equipotent in corn oil and GF. Its potency was substantially lower when administered in Emulphor®, and even lower when given as an aqueous suspension in methylcellulose. Crofton and his co-workers (1995) emphasized the need for TK studies to clarify the reason(s) for the vehicle-dependent differences they observed in acute DLM neurotoxicity.

The overall objective of the current study was to investigate the influence of two common vehicles/diluents on the bioavailability, TK and acute neurotoxicity of orally-administered DLM. An aim of our investigation was to test the hypothesis that DLM dissolved in GF is more acutely neurotoxic to rats than DLM in Alkamuls®, because solubilized DLM is better absorbed from the GI tract and reaches the target organ (brain) in larger quantities.

2. Materials and methods

2.1. Chemicals

Deltamethrin (DLM) [(S)-α-cyano-3-phenoxybenzyl-(1R, *cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)-cyclopropanecarboxylate] (purity, 98.8%) was kindly provided by Bayer CropScience AG (Monheim, Germany). Acetonitrile (HPLC grade) and glycerol formal (GF) were purchased from Sigma-Aldrich (St. Louis, MO). Alkamuls El-620® (formerly Emulphor®) (AL) was a gift from Rhodia (Cranbury, NJ). Methanol, sulfuric acid and deionized water (HPLC grade) were obtained from J.T. Baker (Phillipsberg, NJ). All other chemicals used were of the highest grade commercially available.

GF, a binary solvent, is used to solubilize a wide variety of hydrophobic and hydrophilic chemicals and pharmaceuticals (Sanderson, 1959). It is a condensation product of glycerol and formaldehyde. It is a 60:40 mixture of two chemicals: 4-hydroxymethyl-1,3-dioxolane and 5-hydroxy-1,3-dioxane. GF produces no apparent toxic effects or macroscopic pathology when rats are dosed orally with up to 4000 mg/kg. The only effect manifest at the highest doses is narcosis (Sanderson, 1959). Emulphor® (now Alkamuls®), a polyethoxylated vegetable oil, is widely used to prepare stable aqueous emulsions of aliphatic and aromatic hydrocarbons of low to moderate molecular weight.

2.2. Animal maintenance and preparation

Male, adult Sprague–Dawley (S-D) rats (∼90 days old) were obtained from Charles River Laboratories (Raleigh, NC). The rats were acclimated (2 rats/cage) for at least 10 days in an AAALAC-approved animal care facility maintained at 72 ± 2° F and 50 ± 10% humidity with a 12-h light/dark cycle (light 0600–1800 h). These animals were housed in polycarbonate cages lined with sterilized recycle paper bedding (Tek-Fresh®, Harlan TEKLAD, Madison, WI). Food (5001 Rodent Diet®, PMI Nutrition Internat., St. Louis, MO) and tap water were provided *ad libitum*. The experimental protocol was reviewed and approved by the University of Georgia Animal Care and Use Committee.

Groups of rats were cannulated so that serial blood samples could be taken to characterize the time-course of DLM given in GF and in AL. Briefly, each rat was anesthetized by an injection of 0.1 ml/100 g bw of a "cocktail" consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (20 mg/ml), and xylazine hydrochloride (10 mg/ml) (3:2:1, v:v:v). A cannula (PE-50 polyethylene tubing) was surgically inserted into the left carotid artery until its tip rested just above the aortic arch. The cannula was then securely ligated to the artery, passed sc and exteriorized at the nape of the neck. Thus, the animals could not disturb the cannula, but could move about freely once they awakened. Water was provided *ad libitum*, but food was withheld during the 24-h recovery period before dosing. Food was provided 3 h after dosing.

2.3. DLM treatments and sampling

We were limited in the range of DLM doses that could be evaluated by the insecticide's acute toxicity and by its analytical limit of quantitation. Oral dosages ≥20 mg/kg in GF produced salivation, marked tremors and choreoathetosis. DLM was even more neurotoxic when injected iv in GF. Oral and iv doses in GF had to be ≥2 mg/kg, in order to obtain complete time-courses (i.e., uptake and elimination profiles) of the parent chemical in plasma.

In the time-course experiment DLM was administered iv and po either as a solution in GF or as an aqueous suspension in 5% AL. The oral dose of 10 mg DLM/kg was given by gavage in each vehicle in a total volume of 2 ml/kg. The iv doses of 2 and 10 mg DLM/kg in GF and AL, respectively, were injected in a total volume of 0.2 ml/kg into a lateral tail vein. Each animal was dosed between 09:00 and 10:00 h. Their average body weight at the time of dosing was ∼360 g. Serial arterial blood samples of 150 μl were drawn from the left carotid artery cannula and collected in heparinized tubes at the following time-points: 0.02, 0.08, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 60, 72 and 96 h after iv dosing, and 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 60, 72 and 96 h after oral dosing. An equivalent volume of heparinized saline was injected ia via the cannula after each blood withdrawal. Plasma was separated by centrifugation within 30 min of blood collection. DLM concentrations were analyzed immediately as described below by the method of Kim et al. (2006).

A second experiment was performed to delineate the influence of the dosing vehicles on the deposition of DLM in selected tissues. Groups of 3–4 rats were fasted overnight before being gavaged between 09:00 and 10:00 h with 10 mg DLM/kg in GF or AL. Two h post dosing the rats were euthanized by CO_2 asphyxiation. Samples of blood, whole brain, liver, kidney, lung and perirenal fat were collected and processed for DLM analysis as described below.

In a third experiment groups of 3–4 rats received an iv injection of one of the following: 2 mg DLM/kg in GF; 2 mg DLM/kg in AL; or 10 mg DLM/kg in AL. Each solution was injected in a total volume of 0.2 ml/kg into a lateral tail vein. All of the animals were euthanized by CO_2 asphyxiation after 15 min and the lungs removed for analysis of their DLM content by the method of Kim et al. (2006). The purpose of this experiment was to compare the pulmonary deposition of DLM given in the two vehicles.

2.4. Neurotoxicity assessment

Rats in the DLM time-course study were observed during the 96-h blood sampling period for toxic signs. Complex measures of behavioral effects (e.g., functional observational batteries, motor activity in mazes) have been utilized by some investigators to assess some pyrethroids (McDaniel and Moser, 1993; Wolansky et al., 2007). No quantitative biochemical measures of acute pyrethroid neurotoxicity, however, are apparently available. Therefore, we chose to monitor the animals for

the primary clinical signs of acute Type II poisoning: salivation, tremors, choreoathetosis; and death, as observed in previous studies of DLM (Anàdon et al., 1996; Anand et al., 2006b). The severities of these signs were not scored subjectively, but merely observed and recorded.

2.5. Analysis of DLM

DLM in plasma and tissues was quantified by a high performance liquid chromatography (HPLC) method with UV absorbance detection at 230 nm (Kim et al., 2006). Briefly, DLM was separated on a reverse phase Ultracarb 5 ODS column (250 mm × 4.6 mm; 5 μm particle) (Phenomenex, Torrance, CA), protected by a Security Guard® Fusion-RP guard column cartridge (Phenomenex, Torrance, CA) on a Shimadzu HPLC system (LC-10AT pump, DGU-14A degasser, SIL-HT autosampler, SPD-10AV detector) (Shimadzu, Canby, OR). The mobile phase was 80% acetonitrile and 20% sulfuric acid (1%). The flow rate was set at 1.0 ml/min. Most of the DLM in blood was present in the plasma (data not shown). Therefore, plasma was separated from blood and 65 μl of plasma were added to microcentrifuge tubes containing 130 μl of acetonitrile for extraction. These tubes were vortexed for 15 s and centrifuged for 10 min at 2500 × *g* (Beckman Coulter®, Atlanta, GA). Various tissues were homogenized for 10 s in 4 volumes of a mixture of deionized water and acetonitrile (1:1 ratio). Sixty-five microliters of each homogenized tissue were mixed with 130 μl of acetonitrile and centrifuged. Fifty μl of supernatant were injected onto the guard column. The limits of detection and quantitation for plasma and tissues were 0.01 and 0.05 μg/ml, respectively.

2.6. Particle size measurement

Both the GF and AL formulations were viewed soon after preparation at 40X magnification under a phase-contrast microscope (Bausch & Lomb, Rochester, NY) fitted with a calibrated ocular micrometer. No DLM particles were visible in the GF solution. The microscope's stage was moved in a coordinated manner until a total of 1000 DLM particles were measured in the AL formulation. The average and ranges of particle size were then calculated. The particles were photographed using a Pixera Pro600ES camera (Pixera Corporation, Los Gatos, CA) attached to the microscope.

2.7. Data analyses

The maximum plasma concentration (C_{max}) and time to maximum concentration (T_{max}) values were determined by visual inspection of the plasma DLM concentration versus time profile data for each formulation. Other pharmacokinetic parameters were calculated using Winnonlin (ver. 4.1) noncompartmental model analysis (Scientific Consulting, Inc., Cary, NC). Data are reported as means ± SE. The statistical significance ($p < 0.05$) of apparent differences in pharmacokinetic parameters between the two formulations was assessed by Student's *t*-test using Prism (ver. 3.03) (GraphPad Software, Inc., San Diego, CA). A one-way analysis of variance (ANOVA), followed by the Bonferroni Test (Prism 3.03, San Diego, CA) was performed to determine whether DLM concentrations in plasma and different tissues were significantly different ($p < 0.05$) from one another for each formulation. Student's *t*-test was used to assess the statistical significance of vehicle dependent differences in DLM levels in each tissue 2 h after oral administration of 10 mg/kg.

3. Results

3.1. Toxicokinetics of iv DLM in GF versus AL

Plasma elimination curves for rats given 2 and 10 mg DLM/kg iv in GF and AL, respectively, are shown in Fig. 1. Differences in DLM elimination in the two vehicle groups during the initial 2 h post injection can be seen more easily in the inset. As the plasma DLM concentrations were well below its K_m (Anand et al., 2006a), the TK of DLM should be linear in this dosage range. The mean arterial plasma DLM concentration in the GF group 1-min after injection was 26-fold higher than that in the AL group, despite the 5-fold higher dose in the latter group. Plasma DLM levels decreased very rapidly in both vehicle groups for the first 15 min post injection (Fig. 1 Inset). Kinetic analysis confirmed that plasma elimination was biexponential in both vehicle groups. The initial rapid drop was followed by a slow, prolonged decline for the remainder of the 96-h monitoring period in the AL rats. Sequestered DLM particles in their pulmonary capillaries, as described below, apparently acted

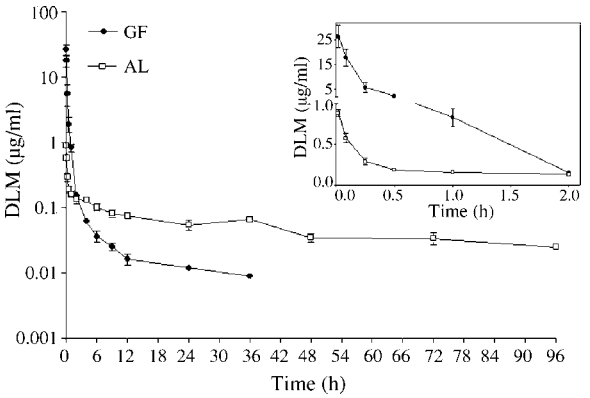

Fig. 1. Plasma concentration-time profiles following iv injection of DLM in GF and AL. Rats received 2 mg DLM/kg in GF or 10 mg DLM/kg iv in AL. Plasma DLM concentrations were measured serially in blood samples taken from a carotid artery cannula from 0.02–96 h post injection. Data points represent means ± SE of groups of 3 or 4 rats. DLM levels were undetectable beyond 36 h in the GF animals. Inset shows DLM levels in the two vehicle groups during the first 2 h. Note the linear scale of the inset's *Y* axis.

Table 1
Toxicokinetic parameter estimates following iv injection of DLM in GF or AL

Toxicokinetic parameters	Glycerol formal (GF) 2 mg DLM/kg	Alkamuls (AL) 10 mg DLM/kg
AUC (μg·h/ml)	7.19 ± 1.77	5.21 ± 1.64
$t_{1/2}$ (h)	15.03 ± 1.31*	30.52 ± 10.60
Vd (l/kg)	7.36 ± 2.70*	85.30 ± 22.45
Cl (l/h)	0.12 ± 0.04*	0.85 ± 0.27

Values represent means ± S.E. of groups of 3 or 4 rats.
* Indicates significant difference ($p < 0.05$) between the two formulations.

as a sustained release dosage form. The terminal elimination half-life ($t_{1/2}$) was twice as long in this group as in the GF group (Table 1). DLM levels in the AL vehicle group exceeded levels in the GF group at all sampling times after 2 h (Fig. 1). As a result, the AL area under the plasma concentration versus time curve (AUC) was not significantly different from that for the GF group (Table 1). The AL volume of distribution (Vd) was markedly higher, due to trapping of DLM particles in the pulmonary microcirculation. This was confirmed by measurement of DLM levels in the lungs 15 min after iv injection of 2 mg DLM/kg in AL and in GF. Concentrations of the insecticide were 10-fold higher in the lungs of the animals receiving the compound in AL (Fig. 2A). Another group given 10 mg DLM/kg iv in AL exhibited an 86-fold higher DLM level than the 2 mg/kg GF group (Fig. 2B).

Plasma uptake and elimination curves for rats gavaged with 10 mg DLM/kg in GF and AL are pictured in Fig. 3. DLM is detectable for 48 h in both vehicle groups. It is obvious that DLM is absorbed from the GI tract to a greater extent when the chemical is administered in GF. The peak plasma level (C_{max}) for this vehicle group is 10.6-fold higher, while the AUC is 9-fold greater (Table 2) than in the AL group. Although bioavailability (F) is 8.8-fold greater, it is still only 15% in the GF animals. As DLM particles in AL were trapped in the pulmonary capillaries, it was necessary to utilize the GF iv data to estimate a F value for the AL group. Interestingly, the shape of the GF and AL uptake and elimination profiles are similar (Fig. 3). Accordingly, several of the TK parameter estimates [e.g., absorption rate constant (ka), time to maximum blood concentration, $t_{1/2}$, Vd and clearance (Cl)] (Table 2) are comparable for the two vehicle groups.

Tissue and plasma DLM concentrations 2 h after oral administration of 10 mg DLM/kg in GF and AL are presented in Fig. 4. DLM concentrations are significantly higher in each biological specimen when the insecticide

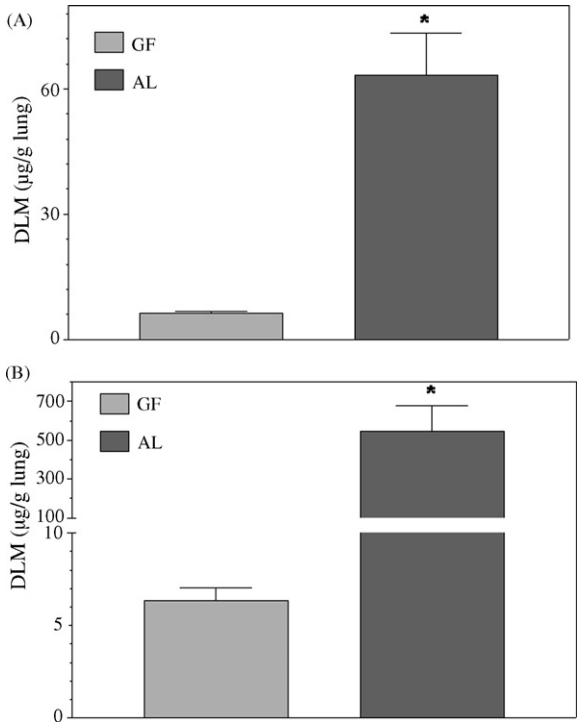

Fig. 2. DLM concentrations in lungs of rats 15 min after iv injection of: [A] 2 mg DLM/kg in glycerol formal (GF) or Alkamuls® (AL); [B] 2 mg DLM/kg in GF and 10 mg DLM/kg in AL. Bar heights represent mean ± SE for groups of 3–4 rats. *Denotes statistically significant difference from 2 mg/kg GF vehicle group.

is given in GF. DLM levels in plasma are substantially higher than in tissues of the GF group 2 h post dosing. Surprisingly, DLM levels are significantly lower in the brain than in plasma and the other tissues in both vehicle groups. Levels in fat are little different from concen-

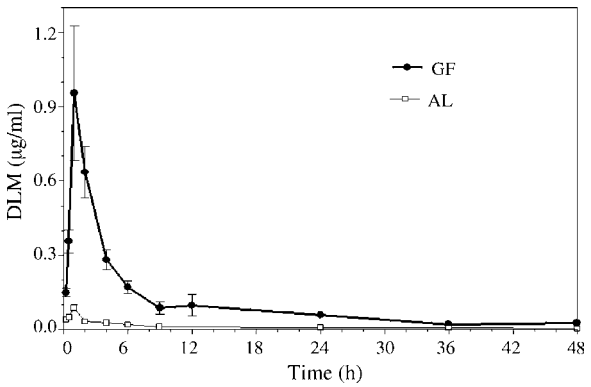

Fig. 3. Plasma concentration-time profiles on linear scales following oral administration of 10 mg DLM/kg in GF or AL. Serial plasma concentrations of DLM were measured over a period of 0.25–96 h. Data points and brackets represent means ± S.E. of 4 or 5 rats. DLM was undetectable beyond 48 h.

Table 2
Toxicokinetic parameter estimates following oral administration of 10 mg DLM/kg in GF or in AL

Toxicokinetic parameters	Glycerol formal (GF)	Alkamuls (AL)
ka (h^{-1})	1.38 ± 0.66	1.60 ± 1.01
C_{max} (μg/ml)	0.95 ± 0.27*	0.09 ± 0.01
T_{max} (hr)	1.50 ± 0.58	1.0 ± 0.00
AUC (μg·h/ml)	5.49 ± 0.22*	0.61 ± 0.14
F	0.15 ± 0.02*	0.017 ± 0.004
$t_{1/2}$ (h)	20.15 ± 3.22	24.82 ± 5.10
Vd (l/kg)	7.99 ± 0.48	8.48 ± 1.17
Cl (l/h)	0.11 ± 0.00	0.10 ± 0.01

Values represent means ± S.E. for groups of 4 or 5 rats.
* Indicates significant difference ($p < 0.05$) between the two formulations. Bioavailability of DLM in AL was calculated using GF iv data, because of substantial pulmonary trapping of DLM given iv in AL.

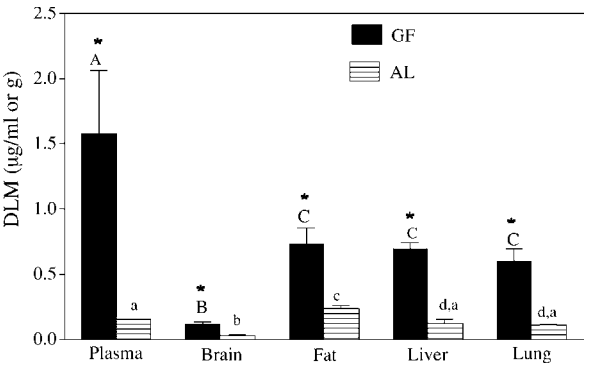

Fig. 4. Plasma and tissue concentrations of DLM 2 h after an oral dose of 10 mg DLM/kg dissolved in GF or suspended in 5% AL. Bars and brackets represent means ± S.E. for 3 or 4 rats. *Denotes DLM concentrations in plasma and each tissue that were significantly higher when the insecticide was given in GF. Different capital and lower case letters designate statistically significant difference within the GF and AL groups, respectively.

trations in the other tissues in either vehicle group at this particular time-point. Comprehensive time-course studies in our laboratory demonstrate that DLM levels in adipose tissue far exceed levels in plasma and other tissues for a prolonged period at later time-points (Mirfazaelian et al., 2006; Kim et al., 2007).

DLM particle size was contrasted in the two vehicles (Fig. 5). The lipophilic chemical appeared to be completely dissolved in GF. In contrast, particles of varying size were visible under a phase-contrast microscope in the AL formulation. The mean particle size was determined to be 10 μm. The size distribution was as follows: <5 μm = 22.2%; >5–12.5 μm = 48.9%; >12.5–25 μm = 20.8%; and >25–50 μm = 8.1%.

Manifestations of acute neurotoxicity were consistent with the aforementioned TK findings. Moderate salivation and slight tremors were evident in rats administered 2 or 10 mg DLM/kg iv and po, respectively, in GF. These signs lasted for 2 to 3 h. No acute neurotoxicity was evident at either dosage level in animals given DLM in AL by either route.

4. Discussion

This investigation demonstrates that oral DLM's greater lethality (Soderlund et al., 2002) and motor depressant activity (Crofton et al., 1995) are due largely to increased GI absorption and bioavailability upon the chemical's dissolution. The systemic, or internal dose of DLM, as reflected by AUC_0^{48} values, was 9-fold higher when the chemical was administered orally in GF rather than AL. The GF group's observed C_{max} was 10.6-fold higher. The concentration of DLM in the whole brain of GF rats was significantly higher than in the brain of the AL rats 2 h post dosing. The animals that received 10 mg DLM/kg in GF exhibited transient salivation and

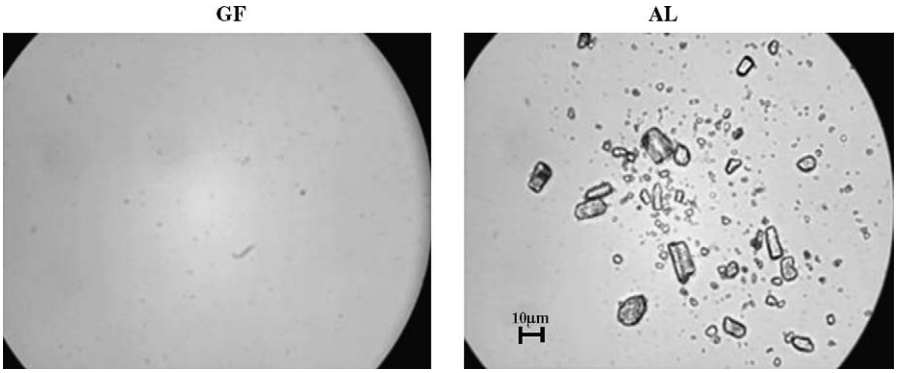

Fig. 5. DLM GF and AL formulations as seen under a phase-contrast microscope (original magnification X 40). Particle size in the AL formulation was measured using a calibrated ocular micrometer attached to the microscope. A 10 μM scale is included to aid in judgement of particle size in AL.

tremors, whereas their AL counterparts were asymptomatic. Thus the oral dosage vehicle, or diluent can have a toxicologically-significant impact on DLM and very likely on other ingested pyrethroids.

Particle size is an important determinant of systemic absorption of hydrophobic drugs and other chemicals from the GI lumen (Jinno et al., 2006; Liversidge and Cundy, 1995). Reducing the size of particulates increases their surface area, enhancing a compound's opportunity for dissolution and diffusion across GI epithelial membranes (Gibaldi, 1984). Jinno et al. (2006) were able to produce microparticles of cilostazol, a hydrophobic drug, that ranged in size from 0.1–100 μm. Bioavailability of the smallest (∼0.1–0.3 μm) NanoCrystals® was about 5-fold higher than NanoCrystals® of ∼0.5–10 μm, when the two formulations were given orally to dogs as an aqueous suspension. The researchers demonstrated that miniaturization of the particles of the highly lipophilic drug significantly increased their solubility in water, and hence their systemic absorption. Damge et al. (1996) measured the uptake of solid microspheres instilled into the ileal lumen of adult rats. Only ∼0.11% of the 5- to 10-μm microspheres were taken up versus ∼12.7% of these 1–5 μm. These researchers observed the microspheres to cross the intestinal mucosa by phagocytotic uptake by specialized membranous cells in the Peyer's patches, lymphoid nodules abundant in the ileum. Nevertheless, it appears that solid nanoparticles of pyrethroids would be taken up in very limited amounts by this mechanism.

Bioavailability and C_{max} in the current study were significantly lower when DLM was given as an aqueous AL suspension than when dissolved in GF. AL has routinely been utilized in our laboratory to prepare stable aqueous emulsions of a variety of volatile organic chemicals that were liquids at room temperature. DLM, however, is a solid (powder). Our AL formulation proved to be an unstable aqueous suspension, in that the DLM particles settled soon after vigorous shaking. Some 22% of the DLM particles in the suspension were <5 μm, but the size distribution of even smaller particles could not be determined. Judging from the aforementioned studies, nanoparticles 0.1–1 μm would best undergo dissolution (and diffusion through GI membranes). Although systemic uptake from our AL suspension was quite limited, T_{max} and ka, two indices of the rate of absorption, were not significantly different between the AL and GF groups. This, it seems reasonable to assume that much less DLM was available for systemic uptake in the AL animals, but that compound which was available (i.e., dissolved) was absorbed from the gut at a comparable rate as that dissolved in GF. Masuh et al. (2000) demonstrated that a substantial reduction of particle size in an aqueous suspension of *cis*-permethrin, a relatively non-toxic pyrethroid, resulted in that formulation having greater insecticidal activity than a commercial suspension of DLM.

DLM and other pyrethroids are marketed in a variety of formulations for application as insecticides. One of the most common DLM preparations is an emulsifiable concentrate (Pawlisz et al., 1998). Some emulsifiable concentrates incorporate the pyrethroid into a petroleum solvent (Mueller-Beilschmidt, 1990). This should dissolve the pyrethroid and result in a true emulsion. Absorption of the dissolved pyrethroid should be more rapid and extensive from the solvent-based product than from aqueous suspensions. Most petroleum solvents themselves quickly diffuse across membranes. The other most common commercial formulation is the flowable suspension concentrate (Pawlisz et al., 1998). It is a dispersion of the powdered insecticide in water. A dispersing agent is included to keep the particles in a deflocculated state. Additional products include a wettable powder, a dustable powder, an aerosol and water-dispersible granules (Tomlin, 1997). The results of the current investigation and of other studies discussed suggest that the oral bioavailability and toxic risks posed to humans by formulations of DLM particulates will be relatively modest, unless the particle size is very small (i.e., <1 μm).

The processes, or mechanisms by which DLM and other pyrethroids are absorbed across the GI epithelium and enter the arterial circulation are not clear. DLM dissolved in GF may be absorbed by passive diffusion into the portal venous blood and be subject to extensive first-pass metabolism by hepatic cytochrome P450s and CaEs (Anand et al., 2006a; Mirfazaelian et al., 2006). This process may account for the low oral bioavailability we found, though it has not been established how efficiently DLM molecules are absorbed. Systemic uptake of DLM from a vegetable oil vehicle would be anticipated to be relatively slow, as the oil should serve as a reservoir in the gut until the lipids are digested. Crofton et al. (1995), however, found DLM given orally in corn oil and GF to have the same time of onset and to be equipotent in reducing motor activity in rats. The oral bioavailability of 14.4% for DLM in sesame oil in rats (Anàdon et al., 1996) is virtually the same as our value of 15% for GF. Long-chain fatty acid micelles from digested triglycerides may carry DLM with them into enterocytes, where they are incorporated into chylomicrons and enter the mesenteric lacteals. Chylomicrons are known to serve as GI carriers for other highly lipophilic, polycyclic hydrocarbons such as DDT (Palin et al., 1982), TCDD

(Lakshmanan et al., 1986) and hexachlorobenzene (Roth et al., 1993). DLM absorbed in this manner from a vegetable oil will bypass first-pass hepatic metabolism, possibly accounting for the comparable bioavailability and bioactivity observed in the aforementioned vegetable oil and GF vehicle groups.

The vehicle-dependent differences we observed in plasma DLM levels were also manifest in organ levels 2 h following oral dosing. In each instance, tissues of rats receiving DLM in GF had significantly higher DLM concentrations than tissues of rats given DLM in AL. It is worthy of note that concentrations of the insecticide in target organ (i.e., brain) were substantially lower than in plasma or the other tissues. The reason(s) for this phenomenon is (are) not clear, though it has been observed with pyrene (Withey et al., 1991) and TCDD (Dilberto et al., 1996) in rats, as well as with 11 chlorinated pesticides and 14 polychlorinated biphenyl congeners in human tissue lipids measured at autopsy in Greenland natives (Dewailly et al., 1999). Although fat DLM levels were not significantly different from liver and lung levels in our rats 2 h after dosing, DLM levels in fat markedly exceeded levels in plasma and in other tissues at later sampling times (Kim et al., 2007; Mirfazaelian et al., 2006). The relatively high DLM concentrations in the lungs 2 h after oral administration can be attributed to the organs' high blood content.

The lungs' high DLM content following iv injection of the compound in AL was due to trapping of DLM particulates in the pulmonary microcirculation. Some 88% of the DLM particulates in AL ranged from >5 to >50 μm. The diameter of pulmonary capillaries varies from 7–10 μm. First-pass pulmonary sequestration of a number of highly lipophilic drugs has been reported following their iv injection. Dutta and Ebling (1998), for example, found peak lung concentrations of propofol, a lipophilic narcotic, to be 300-fold higher when infused iv in a lipid-free medium rather than in a solubilized form.

In summary, the vehicle in which DLM is administered orally has a pronounced influence on the compound's particle size, dissolution, absorption and bioavailability, as well as target and storage tissue deposition. Bioavailability and target organ (brain) levels were significantly higher when the insecticide was given orally to rats in GF rather than as an unstable suspension in AL. Animals receiving 10 mg DLM/kg po in GF exhibited transient salivation and tremors, whereas AL animals were asymptomatic. Persons who ingest solid formulations of DLM are likely to be at a substantially lower risk of neurotoxicity than those who consume the agent in a solubilized aqueous or oil-based formulation. Further toxicity studies will be needed to substantiate this conclusion.

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